Biosensors for intracorporeal measurements–problems and strategies

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Introduction

Electrochemical biosensors and, in particular, enzyme-based sensors have found wide applications in the measurement of specific species in complex media such as biological, industrial and environmental samples. Although relatively limited in number, analytical equipment using such biosensors has been developed in the U.S.A., Europe and Japan and are commercially available for such applications. Since the first publication by Clark & Lyons [1], one of the main challenges for such biosensors has been, and still is, their implantation *in vivo*, either for continuously monitoring metabolites or drugs, especially in intensive care units, or for controlling artificial organs, such as insulin pumps used by diabetic patients [2], or haemodialysis units. For more than a quarter of a century a large number of publications, review, books, workshops and symposia have been devoted to this topic. Seen from outside, no apparent success or improvement has been obtained, since no operating implantable biosensor is presently available. This report will attempt to present some of the real improvements obtained and the various strategies recently developed, mainly illustrated with European examples. Indeed, a biomedical engineering European concerted action (BME-COMAC) has been established since January 1989 on 'Chemical Sensors for *in vivo* Monitoring', under the leadership of A. P. F. Turner.

Abbreviation used: GOx, glucose oxidase.

Biosensors: principles

Biosensors may be variously defined but the name is usually restricted to chemical sensors monitoring

species using biological molecules or reactions for their selective molecular recognition procedure. Thus pH or oxygen sensors, when implanted *in vivo*, are preferably called 'bioprobes' instead of 'biosensors' [3].

All biosensors are analytical devices in which a molecular recognition system is closely associated with or integrated to a system transforming this chemical information into an electric signal [4]: (1) the sensor selectivity is based on a biological molecular recognition system: immobilized or retained enzymes, antibodies or membrane receptors, or biologically integrated systems (plant or animal tissues, micro-organisms); today most implanted biosensors use immobilized enzymes; (2) a physico-chemical transducer or detector monitors the molecular recognition system: it may be electrochemical (potentiometric, amperometric, coulometric, ionic conductivity, surface charge-field effect transistor), thermal or calorimetric (enthalpic) or mass specific (piezoelectric crystal); we will restrict this paper to the first type of detectors, i.e. electrochemical, since they are the most widely used, and, even more specifically, to amperometric enzyme-based sensors.

Operating properties of biosensors

Although the detailed and accurate modelling of biosensors is not always available, their behaviour is generally understood, their rate-limiting steps controlled and their operating parameters well defined. Three types of operating parameters are of importance when these biosensors are used for clinical analysis *in vitro*: (1) analytical parameters characterize their patterns as analytical tools (background signal, sensitivity, linear range, response time, precision, selectivity, sensor life-time and sensor/sample size); (2) signal-controlling parameters may be either physical (local hydrodynamics, membrane permeability, temperature), chemical (pH, buffer capacity, ionic force, cofactor concentration level) or biological (concentration level of molecular recognition species) or, finally, the sample composition itself, i.e. the level of interferents or inhibitors for molecular recognition or transducer reactions; and (3) sensor management methodologies include the calibration procedure but also the evaluation of the above-mentioned analytical parameters.

As these operating parameters may be easily controlled when experiments are made *in vitro*, such devices have proven reasonably reliable. Such evaluation *in vitro* enables the selection of sensors presenting characteristics suitable for each application *in vivo*. Nevertheless, conditions of such evaluation *in vitro* have to be defined in order to approach actual environmental conditions *in vivo*.

Evaluation *in vivo* is even more complex since, besides the choice of animal model, site and method of sensor implantation, procedures have to be found for modifying the metabolite level in such a way that analytical performance of these sensors can be determined accurately during extended periods of operation. These operating parameters may not be directly measurable (for example, background signal, calibration, selectivity, influence of local hydrodynamic conditions, etc.) and specific difficulties may be encountered (e.g. miniaturization, maximum of linear range, site of implantation, clotting on outer membrane, inflammatory and immune reactions to the implant, sterilization procedure, etc.).

Strategies recently developed for *in vivo* glucose sensors

The most widely studied type of implanted biosensor is definitely the glucose one. It is based upon the β-D-glucose oxidation by oxygen in the presence of β-D-glucose oxidase (GOx). Three major strategies have been developed and tested on short-term animal or human experiments [5]: (1) cathodic detection of oxygen depletion by GOx in the presence of glucose, using a specially designed electrode for restricting oxygen partial pressure dependence of the response in blood vessels (Gough *et al.*, San Diego, U.S.A.); (2) anodic detection of hydrogen peroxide produced by GOx in the presence of glucose: after the pioneer work of Shichiri [6], several groups have developed similar strategies (Ege, Copenhagen, DK; Fischer *et al.*, Karlsburg, G.D.R.; Koudelka *et al.*, Neuchatel, Switzerland; Pfeiffer *et al.*, Ulm, F.R.G.; Reach *et al.*, Paris; Vadgama *et al.*, Manchester, U.K.); and (3) anodic detection of GOx reduced by glucose, using ferrocene-type mediators (Pickup *et al.*, London, U.K.).

These strategies may be discussed together with specific problems for *in vivo* glucose sensors: (1) miniaturization of sensors using needle-type geometries suggested by Shichiri *et al.* [6, 7]; (2) deposition of active enzyme layers, using the *p*-benzoquinone covalent immobilization procedure or glutaraldehyde reticulation [7, 8]; (3) choice of the site of implantation, i.e. vascular or subcutaneous [9]; (4) calibration procedure, i.e. determination of background signal and sensitivity *in vivo* [5, 7]; and (5) biocompatibility assessments.

These examples demonstrate the importance of a multidisciplinary approach of *in vivo* chemical sensors and of a tight collaboration between physico-chemists (involved in, for example, analysis, electrochemistry, polymer science) and clinicians (in the fields of diabetology, surgery and biomaterial science) to solve the numerous problems and difficulties encountered. They also show that significant improvements have been obtained allowing short-term *in vivo* implantation, but that very difficult problems have arisen for controlling interactions between biosensor and tissue, i.e. modifications of the sensor by the tissue (e.g. clotting of the external membrane or layer) as well as modifications of the tissue by the sensor (e.g. toxicity, inflammatory and immune reactions), both reactions being usually described as biocompatibility of the implant.

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1. Clark, L. C. & Lyons, C. (1962) Ann. N.Y. Acad. Sci. **102**, 29–45
2. Thévenot, D. R. (1982) Diabetes Care **5**, 184–189
3. Turner, A. P. F. (1987) in Biosensors: Fundamentals and Applications (Turner, A. P. F., Karube, I. & Wilson, G. S., eds.), pp. v–vii, Oxford University Press, Oxford
4. Thévenot, D. R. (1989) Spectra 2000 **17**, 55–57
5. Velho, G., Froguel, Ph., Thévenot, D. R. & Reach, G. (1988) Diabetes, Nutr. Metabolism Clin. Exp. **1**, 227–233
6. Shichiri, M., Yamasaki, Y., Kawamori, R., Hakui, N. & Abe, H. (1982) Lancet **ii**, 1129–1131
7. Sternberg, R., Barrau, M.-B., Gangiotti, L., Bindra, D. S., Wilson, G. S., Velho, G., Reach, G. & Thévenot, D. R. (1989) Biosensors **4**, 27–40
8. Sternberg, R., Bindra, D., Wilson, G. S. & Thévenot, D. R. (1988) Anal. Chem. **60**, 2781–2786
9. Tallagrand, T., Sternberg, R., Reach, G. & Thévenot, D. R. (1988) Horm. Metab. Res. Suppl. **20**, 13–16

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